American Journal of Pathology, Vol. 145, No. 1, July 1994

Evidence for Involvement of ICAM-1 and VCAM-1 in Lymphocyte Interaction with Endothelium in Experimental Autoimmune Encephalomyelitis in the Central Nervous System in the SJL/J Mouse

Bettina J. Steffen, Eugene C. Butcher, and Britta Engelhardt

From the Laboratory of Immunology and Vascular Biology, Department of Pathology, and the Digestive Disease Center, Stanford University School of Medicine, Stanford; and the Center for Molecular Biology in Medicine, Veterans Administration Medical Center, Palo Alto, California

We have investigated the expression of vascular adhesion molecules during the first stage of chronic inflammation in experimental autoimmune encephalomyelitis in the SJL/J mouse. Immunocytochemical analysis of frozen sections of inflamed versus noninflamed brains and spinal cords showed that the vascular endothelium in brains and spinal cords from diseased animals expressed high levels of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) but no detectable mucosal addressin or peripheral lymph node addressin. In frozen section assays, anti-α4 integrin and anti-VCAM-1 monoclonal antibodies inhibited binding of mouse peripheral lymphocytes to inflamed brains at both 4 C and 20 C. Anti-lymphocyte function-associated antigen-1 and anti-ICAM-1 monoclonal antibodies inhibited binding of mouse peripheral lymphocytes to inflamed brains at 20 C. These results are consistent with an important role for the vascular adhesion molecules VCAM-1 and ICAM-1 and for their lymphocyte receptors in lymphocyte recruitment to the central nervous system. (Am J Pathol 1994, 145:189–201)

Mechanisms of leukocyte recruitment to the central nervous system (CNS) during inflammation are poorly understood. In the intact CNS, endothelial cells form the blood-brain barrier (BBB), a tightly interconnected cellular monolayer that strictly controls the free exchange of solute and molecules between the blood and the neuropil. Because of the existence of the BBB and the lack of lymphatic vessels within the CNS, the CNS of mammals has been considered to be an immunologically privileged site.[1] It has been assumed, for example, that the BBB forms an effective barrier for blood cells.[2–4] In contrast to the immune-privileged status of the normal CNS, in human diseases like multiple sclerosis and postinfectious encephalomyelitis and in experimental models of inflammatory diseases of the CNS, immune cells readily gain access to and can be detected in the CNS parenchyma. Contact between the circulating immune cells and the BBB endothelium can be assumed to be the first step leading to CNS invasion. Because the CNS vasculature is structurally unique, it is likely that the inflammatory infiltrate is in part controlled by the induction or upregulation of expression of specific cell adhesion molecules (CAMs) on the activated BBB endothelium.

To explore the vascular mechanisms involved in CNS inflammation, we chose to study experimental autoimmune encephalomyelitis (EAE) in the SJL/J mouse, a model that has been well characterized immunologically, clinically, and pathologically.[5,6] EAE is an inflammatory demyelinating disease of the central nervous system. Because of its histopathological and clinical characteristics it is considered to be the pro-

Supported in part by NIH grants GM 37734 and GM 45448.

BE was funded by the Deutsche Forschungsgemeinschaft, grant En214/2-1. BJS was funded by the Stanford Medical School Scholar Fund.

Accepted for publication March 2, 1994.

Address reprint requests to Britta Engelhardt, PhD, Max-Planck-Institut für physiologische und klinische Forschung, W.G. Kerckhoff-Institut, Abteilung molekulare Zellbiologie, Parkstraße 1, 61231 Bad Nauheim, Germany.

totypical model for inflammatory demyelinating diseases of the human CNS such as multiple sclerosis.[7,8,9] One target autoantigen in EAE is myelin basic protein (MBP), a structural protein of the CNS myelin sheath.[10] EAE is mediated by activated MBP-specific $CD4^+$ T cells.[11] These autoaggressive $CD4^+$ T lymphocytes are responsible for the cellular events leading to edema, inflammation, and demyelination within the CNS white matter.[6,9] However, labeling studies have shown that the MBP-specific $CD4^+$ T cells comprise only a minority of the chronic inflammatory infiltrate in the CNS,[12–15] suggesting that most inflammatory cells are recruited in an antigen-independent way during the inflammatory response. Recent studies therefore focus on the investigation of CAMs that mediate the interaction between circulating cells and the cerebral endothelium.

Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), both members of the immunoglobulin gene superfamily, have been implicated in the adhesion of inflammatory cells to inflamed endothelium.[16–18] Mucosal addressin (MAdCAM-1), a member of the same family, mediates selective homing of lymphocytes to the mucosal-associated lymphoid tissue.[19] The expression of all three molecules can be induced *in vitro* and *in vivo* by stimulation with an overlapping array of proinflammatory cytokines such as interleukin-1 (IL-1) tumor necrosis factor-α, interferon-γ and bacterial endotoxin (LPS) (reviewed in refs. 20, 21). ICAM-1 mediates adhesion of leukocytes to endothelium via their β2-integrin lymphocyte function-associated antigen-1 (LFA-1), and of monocytes and neutrophils via Mac-1.[22] VCAM-1 can mediate the adhesion of mononuclear cells to endothelium via two ligands of the integrin family, namely the $\alpha 4\beta 1$-integrin (VLA-4) expressed on lymphocytes, monocytes, and eosinophils,[23] and activated $\alpha 4\beta 7$-integrin, expressed on gut-tropic lymphocytes.[19,24] In its resting stage $\alpha 4\beta 7$-integrin is a ligand for the mucosal addressin MAdCAM-1.[19] A role for α4-integrins in homing to the CNS has been implicated in EAE by recent studies.[25,26] Whereas VCAM-1 seems to be a likely endothelial ligand during initiation of EAE,[26] the nature of the vascular ligand during the first clinical episode has not been clearly defined.[25,26] Additionally, there is some evidence that ICAM-1 probably expressed on the encephalitogenic T cell might play a role in the development of EAE.[26,27] MAdCAM-1 expression has only been reported at established sites of inflammation in the CNS during the chronic relapsing phase of EAE.[28] A functional role of MAdCAM-1, however, has not been demonstrated.

The present study was undertaken to examine the expression and function of vascular cell adhesion molecules for leukocytes in the central nervous system (CNS) during the first clinical episode of experimental autoimmune encephalomyelitis in the SJL/J mouse. The expression of CAMs in the CNS was investigated by immunostaining frozen sections of brains and spinal cords of SJL/J mice afflicted with EAE. We used monoclonal antibodies (MAbs) directed against a wide variety of endothelial and leukocyte CAMs that have been shown previously to play a role in leukocyte recruitment to different organs.

By performing adhesion assays on frozen sections of brains the functional importance of these CAMs for the recruitment of inflammatory cells to the CNS was further investigated.

Materials and Methods

Mice

Female SJL/J mice were obtained from Jackson Laboratories (Bar Harbor, ME) between 5 to 10 weeks of age. Female BALB/c mice were obtained from the breeding facility of the Palo Alto Veterans Administration Medical Center Animal Research Facility. Mice were maintained at the animal research facility.

Induction of EAE

Active EAE (aEAE) was induced by immunizing SJL/J mice with different doses (2.5 mg, 5.0 mg, 10.0 mg and 25.0 mg) of spinal cord homogenate from syngeneic mice in complete Freund's adjuvant (CFA) (GIBCO BRL, Grand Island, NY), containing 6 mg/ml *Mycobacterium tuberculosis* H37Ra and 10 mg/ml *M. butyricum* (Difco Laboratories, Detroit, MI) into hind footpads. 3×10^9 organisms of heat-killed *Bordetella pertussis* (kindly provided by Dr. David Relman, Stanford University) were injected in 0.5 ml of phosphate-buffered saline (PBS) on days 1 and 3 after immunization. For passive transfer of EAE (tEAE), donor SJL/J mice were immunized with 50 µg guinea pig myelin basic protein (gpMBP) in CFA into the hind footpads. Draining lymph nodes were removed 10 days later. A lymph node cell suspension was prepared and cultured (1×10^7 cells/ml) in RPMI 1640 (Applied Scientific, San Francisco, CA) supplemented with 10% fetal calf serum, penicillin G (100 U/ml), streptomycin (100 mg/ml), L-glutamine (2 mmol/L), sodium pyruvate (100 mmol/L), nonessential amino acids (1%) (all additives Applied Scientific), β-mercaptoethanol ($2, 5 \times 10^{-5}$ M, Sigma Chemical Co., St. Louis MO), and gpMBP at 10 µg/ml. Cells

were cultured for 4 days at 37 C and 10% CO_2. For adoptive transfer of EAE cells were washed, and cell number and viability were assessed by trypan blue exclusion. Between 2 and 5 $\times$ 10^7 viable cells in Hank's balanced salt solution (HBSS, Applied Scientific) were injected into a tail vein of a syngeneic recipient animal. Animals were checked daily for signs of clinical disease. Animals were sacrificed when they exhibited a clinical score of either 1 (limp tail) or 2 (hindleg weakness). Clinical disease occurred approximately 21 days after immunization with spinal cord homogenate and 14 days after transfer of gpMBP-stimulated cells.

Monoclonal Antibodies

The following rat hybridomas were obtained from American Type Culture Collection (Rockville, MD): 30G.12 (anti-murine CD45), GK1.5 (anti-murine CD4), Lyt-2 (anti-murine CD8), M1/70 (anti-murine CD11b), FD441.8 (anti-murine CD11a). The rat hybridomas RA3-6B2 (anti-murine CD45R, Ly5) and RA6-8C5 (anti-murine granulocyte) were a generous gift from Dr. B. Coffman, DNAX, Palo Alto, CA. The rat hybridoma R1.2 (anti-murine CD49d) was kindly provided by Dr. I. Weissman (Stanford, CA),[29] and YN1/1.7 (anti-murine ICAM-1) was a generous gift of Dr. F. Takei (Vancouver, British Columbia, Canada). Rat MAbs PS/2 (anti-murine CD49d), MK2.7 and MK1.9 (anti-murine VCAM-1) were generously provided by Dr. P.W. Kincade (Oklahoma City, OK).[30,31] MK2.7 inhibits α4-integrin mediated adhesion to VCAM-1, whereas MK1.9 does not. 23G.2 (anti-murine CD45RB) was a gift from Dr. E. Pure (Dallas, TX).[32] EA-1 (anti-murine α6-integrin) was received from Dr. B. Imhof (Basel, Switzerland).[33] M290 (anti-murine αE-integrin),[34] M301 and M298 (anti-murine β7-integrin)[35] were a gift from Dr. P. Kilshaw (Cambridge, England). HP2/1 (anti-human CD49d) was kindly provided by Dr. F. Sanchez-Madrid, Madrid, Spain).[36] 6.5E (anti-human CD18), MJ 64 (anti-murine CD44), Mel-14 (anti-murine L-selectin),[37] MECA-79 (anti-murine peripheral lymph node addressin),[38] MECA-367, MECA-89 (both anti-murine mucosal addressin, MAdCAM-1),[39] LS 722 (anti-murine β7-integrin,[21] Fib22 (anti-murine β7-integrin), (Andrew et al., submitted), DATK 32 (anti-murine α4β7),[40] MECA-325 (anti-murine high endothelial venules),[41] MJ 7/18 (anti-murine endoglin, Ge A, and Butcher EC, submitted), and Hermes-1 (anti-human CD 44, used as an isotype-matched control),[42] were raised in our own laboratory.

Cell Lines

The T lymphoma cell line Jurkat (human acute T cell leukemia) was a gift from Dr. Ted Yednock (Athena Neurosciences, South San Francisco, CA). The human monocyte cell line U937 was obtained from American Type Culture Collection. T lymphoma cell line TK1 (AKR mouse T cell lymphoma)[43] was a generous gift from Dr. I. Weissman. Mouse mesenteric and peripheral lymph node cells were isolated from naive BALB/c mice. Activated mouse peripheral lymph node cells were obtained from draining lymph nodes of BALB/c mice immunized with 50 µl CFA in the footpads 10 days before use.

Immunocytochemistry

At signs of clinical disease, animals were anesthetized using Methoxyflurane anesthesia (Metofane, Pitman Moore, Denver, CO), and were perfused with 1% paraformaldehyde in PBS through the left ventricle of the heart. Spinal cord and brain were removed, embedded in Tissue-tec ornithine carbamoyltransferase (OCT, Miles, Inc., Elkhart, IN), and snap-frozen in a 2-methylbutane (EM Science, Gibbstown, NJ) bath at −80 C. Cryostat sections (6 µ) were air-dried overnight, acetone-fixed, and stained using a three-step immunoperoxidase technique. Sections were incubated sequentially with primary MAbs, biotinylated secondary goat-anti-rat IgG (Kirkegaard & Perry Labs, Gaithersburg, MD), and horseradish peroxidase-conjugated Streptavidin (Kirkegaard & Perry Labs) for 30 minutes each step in a humidified chamber, with PBS washes in between the single steps. Sections were developed with 0.07% amino-ethylcarbazol (AEC, Sigma) and 0.009% hydrogen peroxide in 0.01 mol/L acetate buffer (pH 5.2) for 10 minutes. Sections were counterstained with Hematoxylin and Eosin (Gill's formula, Fisher Scientific, Fair Lawn, NJ), coverslipped with Aquamount (Lerner Laboratories, Philadelphia, PA), and immediately analyzed.

Adhesion Assays

Animals were anesthetized using Methoxyflurane anesthesia (Metofane, Pitman Moore). Brain tissue was removed, embedded in OCT (Miles, Inc.) and snap-frozen in a 2-methylbutane (EM Science, Gibbstown, NJ) bath at −80 C. Cryostat sections (10 µ) were air-dried for 1 hour. Frozen-section assays have been established by Stamper and Woodruff.[44] Several modifications were made to their original protocol in

order to conduct frozen-section assays on brain sections. Cells or sections were preincubated with 10 μg/ml MAb in Dulbecco's modified Eagle's medium, 25 mmol/L HEPES (Sigma) and supplemented with 5% bovine calf serum for 20 minutes at 4 C or for 10 minutes at 20 C. Sections were co-incubated with 1 × 10^6 T lymphoma cells or 5 × 10^6 mouse lymphocytes in 100 μl for 30 minutes at 4 C under shear or with 3 × 10^6 mouse lymphocytes in 100 μl for 20 minutes at 20 C under shear.

Naive SJL/J brain sections were used as controls. Assays were fixed in 2.5% glutaraldehyde in PBS for 2 hours. The sections were washed twice in PBS, stained with toluidine blue, and immediately observed.

Because of the high quality of the frozen sections necessary for these adhesion assays, it was not possible to perform each assay on serial brain sections. We therefore performed a semiquantitative analysis of these assays, according to the following method. Control sections had to meet a minimum requirement of at least five vessels with five to seven bound cells/vessel; otherwise the entire assay was not scored. Assays were repeated until a minimum of five assays could be scored for each cell type at each temperature. Each assay was additionally evaluated by a blinded observer. Within one scored assay, sections were assigned to three categories depending on the number of vessels present in the section, and the number of bound cells per vessel relative to the control preparation: (+) or no inhibition, (+/−) or partial inhibition, and (−) or complete inhibition. Results represent the summary of at least five assays per described condition.

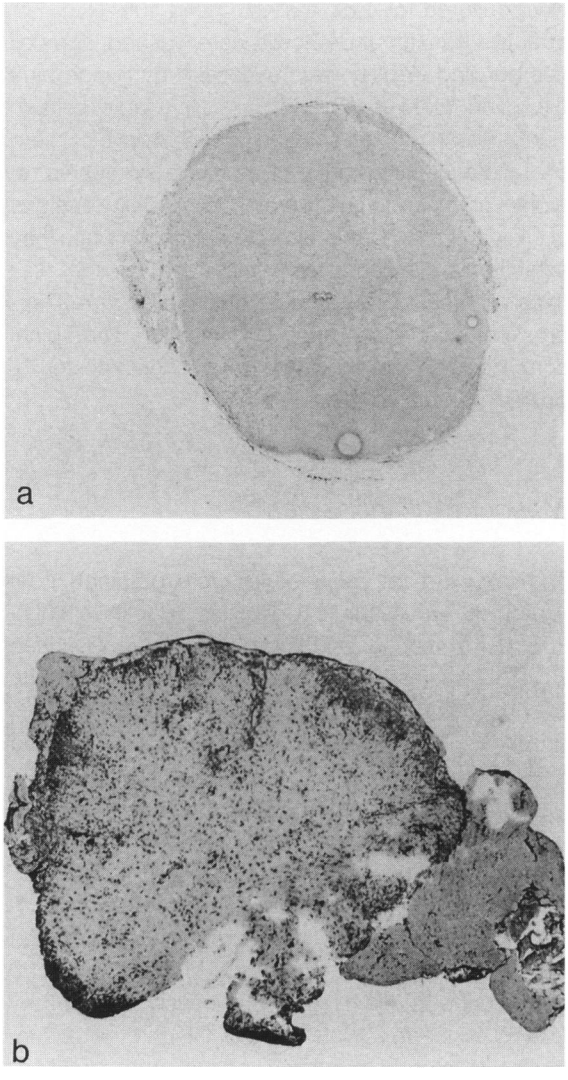

Figure 1. *Inflammatory cell infiltrate in the CNS. Spinal cord of control* (a) *and tEAE mouse* (b). *Immunoperoxidase staining with MAb 30G.12; hematoxylin-eosin counterstain;* ×*40.*

Results

Localization and Phenotype of Cellular Infiltrate

Inflammatory cells (ICs) were identified in frozen sections of brain and spinal cord tissue by staining with the MAb 30G.12, an anti-mouse CD45. No $CD45^+$ cells could be demonstrated in the CNS parenchyma of control mice. Scattered perivascular cells, probably perivascular microglial cells, stained faintly with 30G.12. In sections of spinal cords and brains of diseased animals, massive infiltrates of $CD45^+$ cells were present (Figure 1). ICs accumulated around venules in the CNS white matter forming inflammatory cuffs, one of the characteristic histopathological features of EAE. Brain parenchymal infiltrates were found primarily in periventricular regions. Immunocytochemical characterization of the IC phenotype showed predominant $CD4^+$ T-cell and $CD11b^+$ macrophage populations stained brightly by MAb GK 1.5 and MAb M1/70, respectively. Few $CD8^+$ T cells and few B cells were detected in the infiltrate within the CNS parenchyma.

Neutrophils were characterized by intense RB6-8C5 staining and were observed primarily in meningeal infiltrates and rarely in the parenchymal infiltrates of either the spinal cord or brain. These results are comparable to those of other researchers who have investigated the infiltrating cells in SJL/J mouse EAE.[9,12,14,15,45–47]

Our next interest was to phenotypically characterize the ICs further with regard to their expression of CAMs, as it has been shown before that phenotypically distinct lymphocyte subpopulations defined by

their patterns of "homing receptor" or CAM expression can migrate in a tissue-specific way.[48]

Confirming the data of Zeine and Owens[49] most lymphocytes present in the CNS of mice afflicted with EAE stained very weakly for CD45RB, a marker that is highly expressed on virgin T cells, and downregulated on memory or activated effector T cells.[32] Furthermore, most lymphocytes stained intensely for CD44 and LFA-1 (Figure 2) and expressed ICAM-1, which is characteristic for memory cells.[50]

All ICs stained intensely for α4-integrin using the MAb PS/2 (Figure 2). To date the α4 integrin is known to form heterodimers either with the β1-integrin chain (VLA-4) or with the β7-integrin subunit. Only scattered ICs within the CNS parenchyma expressed β7-integrin, demonstrated by staining with a variety of anti-murine β7-integrin MAbs (M310, M298, LS 722, Fib 22, Figure 2). As no anti-murine β1-integrin MAb was available to us, we were unable to assess directly the presence of β1-integrin on the ICs. However, given the general lack of staining with anti-β7-integrin MAbs and the additional lack of staining with the MAb DATK 32, which is an MAb that recognizes β7-integrin only in context with α4-integrin,[30] we would like to conclude that ICs express the α4β1-integrin heterodimer (VLA-4). A few ICs stained with the MAb M290 directed against murine αE-integrin, which is known to form a heterodimer with β7-integrin. In serial sections the localization of αE- and β7-integrin-expressing ICs was similar, suggesting that these cells might express the αEβ7-integrin heterodimer. No ICs stained detectably for α6-integrin, expression of which has been correlated with the migration of memory T lymphocytes to inflamed skin.[48] None of the ICs located in the parenchymal infiltrates stained for L-selectin, although scattered cells stained intensely for L-selectin could be found in meningeal infiltrates (data not shown).

Expression of CAMs on Endothelium

We investigated the endothelial expression of CAMs in the spinal cords and brains of diseased animals during the first stage of inflammation, and compared them to expression patterns in the CNS of naive syngeneic animals.

We initially investigated the expression of endothelial ICAM-1 and VCAM-1. The lack of a suitable reagent made it impossible for us to investigate the expression of E-selectin. Using MAb YN1/17, we detected constitutive expression of ICAM-1 on large venules in spinal cords and brains of naive SJL/J mice (Figure 3). During the first clinical episode of aEAE, ICAM-1 was upregulated on these venules in spinal cord and brain tissue, and was additionally induced on capillaries. The endothelial expression of ICAM-1 was not limited to vessels associated with cellular infiltrates, but could be detected throughout the entire CNS. Surprisingly, the same expression pattern of endothelial ICAM-1 could be detected in the CNS of control mice, injected intravenously solely with attenuated *B. pertussis* organisms. In tEAE, ICAM-1 expression was upregulated on venules throughout the

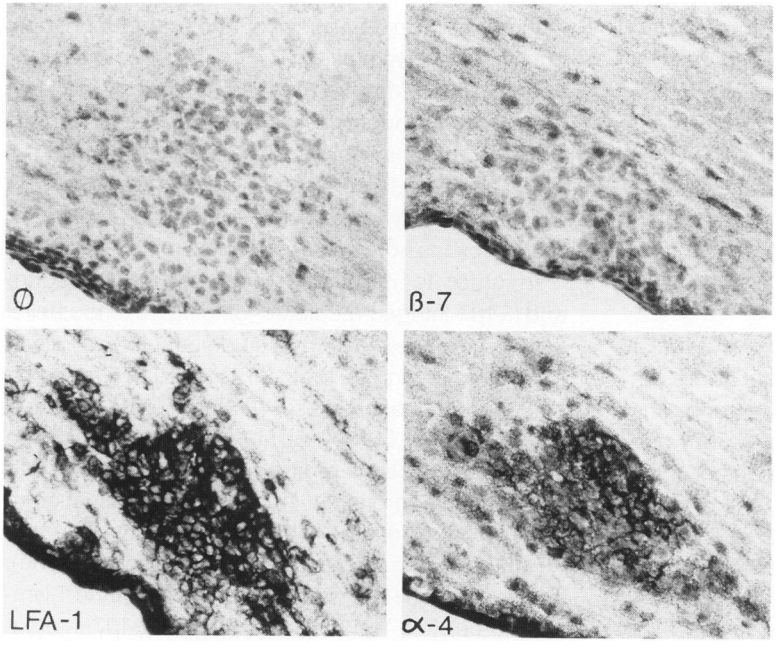

Figure 2. *Expression of CAMs on inflammatory cell infiltrate in the spinal cord of an aEAE mouse (day 24 after immunization). All inflammatory cells express LFA-1 (MAb FD441.8) and α4-integrin (MAb PS/2). Only scattered cells express β7-integrin (Fib22). Ø represents an isotype-matched control (mAb Hermes-1). Immunoperoxidase staining, hematoxylin-eosin counterstain; ×240.*

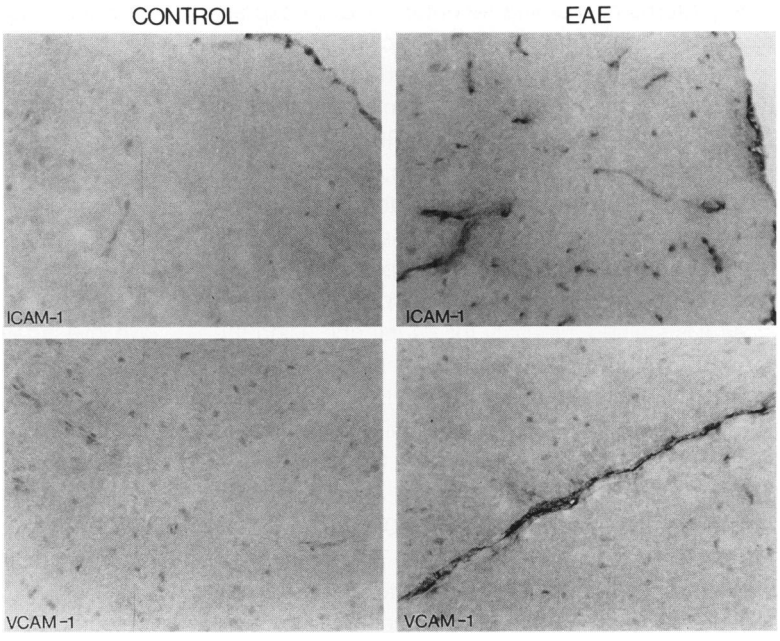

Figure 3. *Expression of vascular cell adhesion molecules in the CNS. Constitutive expression of ICAM-1 on venules in the brain of a naive SJL/J mouse (control, ICAM-1). Endothelial ICAM-1 expression is upregulated in the brain of an aEAE SJL/J mouse on large venules and capillaries (EAE, ICAM-1). Note the absence of ICs surrounding the intensely stained endothelium. Constitutive weak expression of VCAM-1 on large venules in a brain of a naive SJL/J mouse (control, VCAM-1). Upregulation of endothelial VCAM-1 on a large venule in an aEAE brain (EAE, VCAM-1). Immunoperoxidase staining with YN1/1.7 and MK2.7; hematoxylin-eosin counterstain; ×120.*

CNS, and was, as in the aEAE model, not limited to sites of IC infiltrates. In contrast to aEAE, however, endothelial ICAM-1 could not be detected on MECA-99^+ capillaries of animals with tEAE. This suggests that endothelial expression of ICAM-1 might be induced by *B. pertussis* organisms alone.

Using the MAbs MK2.7 and MK1.9, which recognize different epitopes of murine VCAM-1, weak expression of VCAM-1 could be demonstrated on large venules in spinal cords and brains of naive SJL/J mice. In both EAE models, VCAM-1 expression was upregulated on venules in brains and spinal cords and like the ICAM-1-expression was not limited to sites of cellular infiltrates (Figure 3). In contrast to the above-mentioned upregulation of ICAM-1, *B. pertussis* organisms alone did not upregulate the expression of VCAM-1. Endothelial upregulation of ICAM-1 and VCAM-1 was shown to be restricted to the CNS, as neither molecule was induced or upregulated on venules in uninvolved tissues such as the heart, thymus, kidney, peripheral lymph node, mesenteric lymph node, spleen, Peyer's patches, or liver (data not shown).

We also investigated the expression of the vascular addressins, the peripheral lymph node addressin (PNAd, defined by MAb MECA-79), and the MAdCAM-1 (defined by MAbs MECA-367 and MECA-89). We could not detect any endothelial expression of PNAd or MAdCAM-1 in the CNS of SJL/J mice during their first clinical episode of either aEAE or tEAE. Another high endothelial venule- (HEV)-associated antigen, recognized by mAb MECA-325, could also not be detected on any vessel in either the spinal cord or brain of diseased animals during the first inflammatory phase of EAE.

Function of CAMs in Adhesion

In order to investigate the interactions of lymphocytes with endothelial ICAM-1 and VCAM-1 in brains with EAE, we performed binding assays on unfixed frozen sections of brains, using a modified protocol of the originally described frozen section assay, by Stamper and Woodruff.[44] Because of the difficulty involved in removing nonfixed spinal cord tissue of mice in a manner in which the assay could be performed, only brain sections were used. The expression patterns of endothelial CAMs and the time kinetics of their upregulation were identical in brains and spinal cords of EAE-afflicted animals, and, in addition, the brain tissue provided a larger tissue area with more vessels on which to study lymphocyte adhesion. As a control, brain sections from naive syngeneic mice were used.

In order to distinguish between $\alpha 4\beta 1$-integrin- and $\alpha 4\beta 7$-integrin-mediated binding to endothelial VCAM-1, two cell lines were studied for their ability to bind to vessels in EAE brains. The human T lymphoma cell line Jurkat expresses $\alpha 4\beta 1$-integrin (VLA-4) and the $\beta 2$-integrin LFA-1 but no $\alpha 4\beta 7$-integrin and no CD44. Jurkat cells are known to bind via VLA-4 to VCAM-1 and fibronectin (T. Yednock, personal communication). Jurkat cells do not bind to ICAM-1 or to MAdCAM-1 (M. Briskin, personal communication). The second cell line we tested was the murine T lymphoma cell line TK1, which expresses $\alpha 4\beta 7$-integrin

in addition to LFA-1, but not $\alpha 4\beta 1$-integrin. TK1 cells bind *in vitro* via their $\alpha 4\beta 7$-integrin to MAdCAM-1[19] and after activation to VCAM-1 and fibronectin.[24]

Adhesion assays were conducted at 4 C to compare the binding via $\alpha 4\beta 1$-integrin and $\alpha 4\beta 7$-integrin. Jurkat cells bound selectively to venules exposed in frozen sections of EAE brains, but not to any vessels in sections of control brains (Figure 4).

Neither pre-incubation of Jurkat cells with anti-human CD44 (MAb Hermes-1) or with anti-LFA-1 antibody (MAb 6.5E, anti-CD18) nor pre-incubation of brain sections with anti-ICAM-1 antibody (MAb YN1/1.7) had any effect on the ability of Jurkat cells to bind to vessels in EAE brains. In contrast, pre-incubation of Jurkat cells with anti-$\alpha 4$-integrin antibody (MAb HP2/1) completely inhibited their adhesion to vessels of EAE brains. Similarly, pre-incubation of brain sections with anti-VCAM-1 antibody (MAb MK 2.7) completely abolished binding of Jurkat cells to inflamed cerebral vessels (Figure 4).

TK1 cells also bound selectively to inflamed vessels exposed on sections of EAE brains, but not to vessels on control brain sections (data not shown). Pre-incubation of tissue sections with anti-VCAM-1 antibody (MAb MK 2.7) completely inhibited binding of TK1 cells to inflamed vessels, suggesting endothelial VCAM-1 to be a ligand for TK1 binding. Pre-incubation with anti-$\alpha 4$-integrin antibody (MAb PS/2) inhibited binding significantly (Table 1), and anti-$\beta 7$ MAb Fib 22 and anti-$\alpha 4\beta 7$-integrin MAb DATK 32

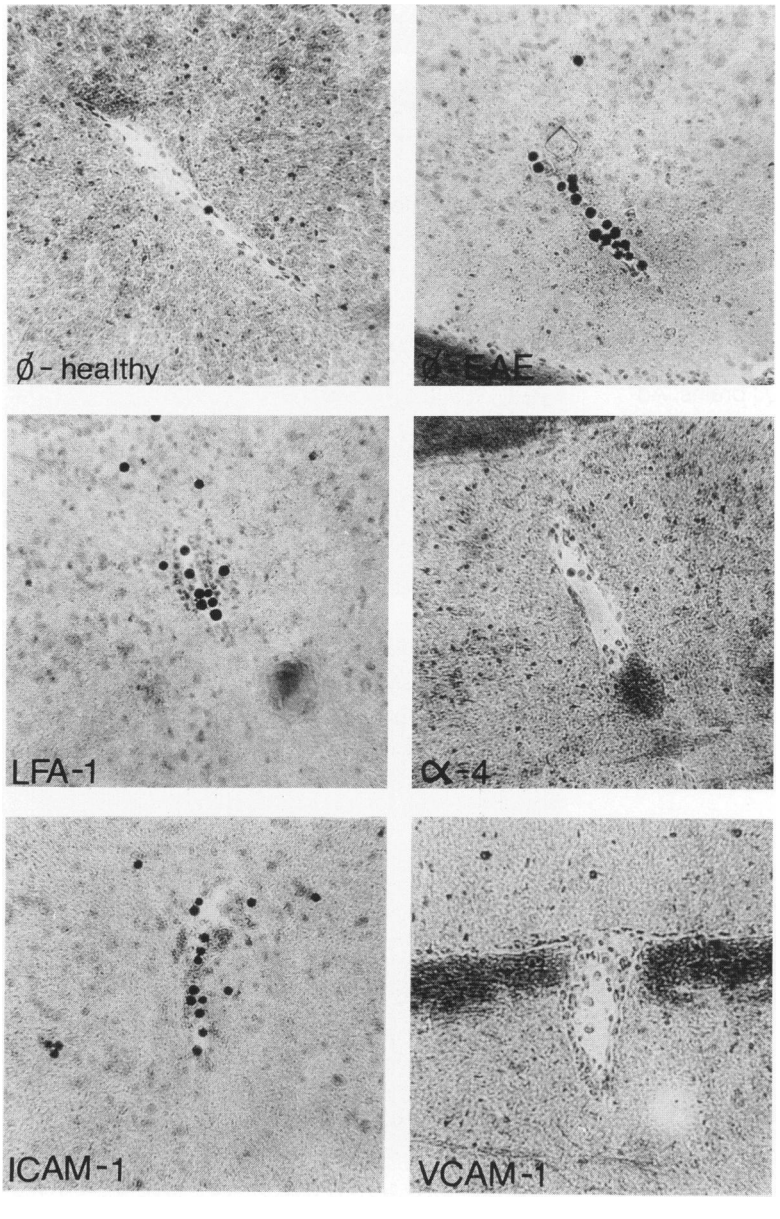

Figure 4. *Adhesion of Jurkat cells to inflamed vessels in EAE brain at 4 C. Jurkat cells bind poorly to the endothelium of vessels in naive SJL/J brains (Ø, healthy). Selective adhesion of Jurkat cells to inflamed venules in an EAE brain (Ø-EAE). Preincubation with MAb Hermes-1 (anti-human CD44) has no effect on binding of Jurkat cells (not shown). Nearly complete inhibition of binding of Jurkat cells to the inflamed endothelium in EAE brains by MAb HP2/1 (anti-human α4-integrin) or by MAb MK2.7 (anti-murine VCAM-1). MAbs directed against LFA-1 (6.5E) and ICAM-1 (YN1/1.7) show no effect. Toluidine blue staining; ×160.*

Table 1. *Semiquantitative Summary of Adhesion Assays*

	Jurkat (4 C)	TK1 (4 C)	SLN* (4 C)	(20 C)
No MAb	+†	+	+	+
Isotype control‡	+	+	+	+
Anti-VCAM-1	–	–	–	±
Anti-α4	–	±	–	±
Anti-ICAM-1	+	+	±	–
Anti-LFA-1	+	+	±	–
Anti-β7	nd	–	+	nd
Anti-α4β7	nd	–	+	nd

* *In vivo* by CFA injection stimulated lymph node cells.
† + = adhesion or no inhibition; ± = partial inhibition; – = complete inhibition; nd = not done.
‡ MAb used were as follows: isotype control = Hermes-1; anti-VCAM-1 = MK2.7; anti-α4 = PS/2; anti-ICAM-1 = YNI1/1.7; anti-LFA-1 = FD441.8/6.5E; anti-β7 = M298; anti-α4β7 = DATK 32.

completely blocked the interaction. Pre-incubation of cells with anti-LFA-1 antibody (MAb FD 441.8) and pre-incubation of brain sections with anti-ICAM-1 antibody (MAb YN1/1.7) had no effect on binding of TK1 cells at 4 C. These data suggest that TK1 cells use α4β7-integrin to bind to endothelial VCAM-1 in the inflamed CNS venules.

We further tested the ability of lymph node cells to bind to inflamed vessels. Naive lymph node cells did not bind to vessels in either control or EAE-afflicted brains. However, lymph node cells isolated by subcutaneous injection of CFA-stimulated lymph nodes selectively bound to inflamed vessels in EAE brains but not to vessels exposed in naive SJL/J brains. Adhesion assays were conducted at 4 C and at 20 C. Stimulated lymph node cells (SLN) selectively bound to vessels in EAE brains at both temperatures. At 4 C, binding of lymph node cells could be completely inhibited by pre-incubation of cells with antibodies directed against α4-integrin (MAb PS/2, Table 1, Figure 5) or by pre-incubation of brain sections with anti-VCAM-1 antibody (MAb MK 2.7). Antibodies against β7-integrin had no effect. Anti-LFA-1 (MAb FD441.8) and anti-ICAM-1 (MAb YN1/1.7) inhibited their binding partially (Table 1, Figure 5). Thus, at 4 C SLN could bind to cerebral vessels via α4β1-integrin with a contribution of LFA-1.

At 20 C, the ability of MAbs directed against either LFA-1 or ICAM-1 to interfere with binding of SLN to inflamed cerebral vessels was dramatically changed (Table 1). At this temperature, antibodies against α4-integrin (MAb PS/2) or VCAM-1 (MAb MK 2.7) inhib-

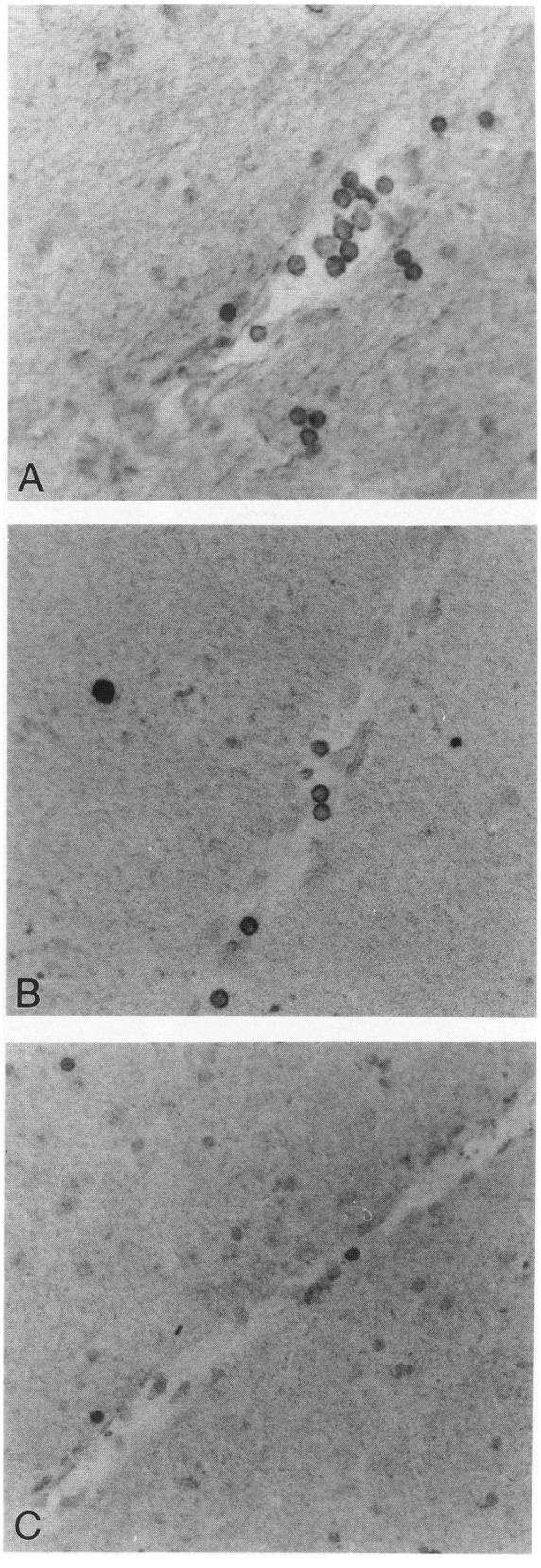

Figure 5. *Adhesion of stimulated mouse peripheral lymph node cells to inflamed endothelium in EAE brains at 4 C. Selective adhesion of lymph node cells to the endothelium in EAE brains* (**A**). *Partial inhibition of lymph node cell adhesion to inflamed cerebral vessels with MAb FD441.8 (anti-LFA-1,* **B**). *Pre-incubation of lymph node cells with MAb PS/2 (anti-murine α4-integrin) completely inhibits binding* (**C**). *Toluidine blue staining; ×300.*

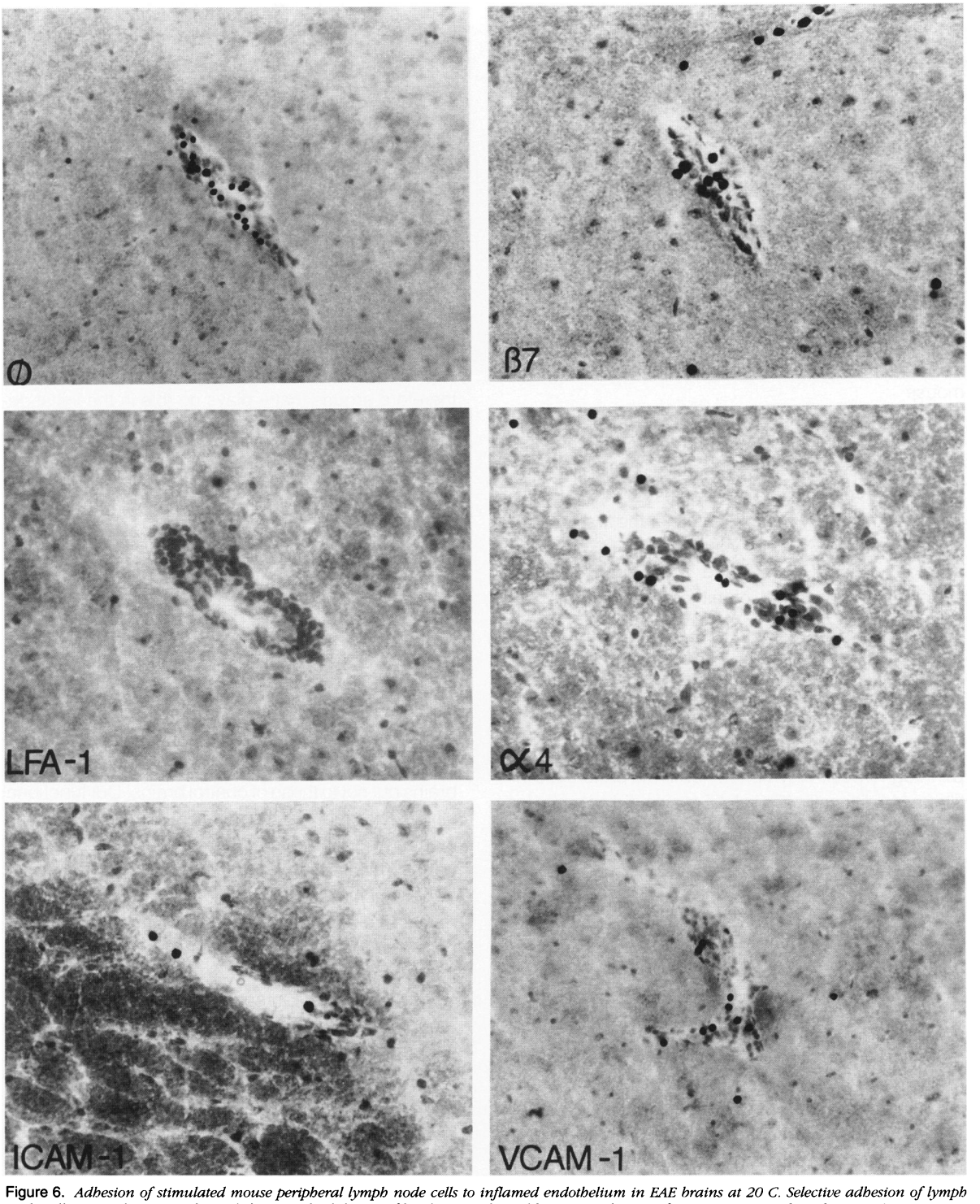

Figure 6. *Adhesion of stimulated mouse peripheral lymph node cells to inflamed endothelium in EAE brains at 20 C. Selective adhesion of lymph node cells to vessels in aEAE brain (Ø). Partial inhibition of binding is achieved by pre-incubation of tissue sections with MAb MK2.7 (anti-murine VCAM-1) or lymph node cells with PS/2 (anti-α4-integrin). Almost complete inhibition of binding of lymph node cells by pre-incubation of tissue sections with MAb YN1/1.7 (anti-ICAM-1) or lymph node cells with FD441.8 (anti-LFA-1). Toluidine blue staining; ×160.*

ited binding of SLN cells to venules in EAE brains, but to a lesser degree than at 4 C (Figure 6). Pre-incubation of the SLN with anti-LFA-1 (MAb FD441.8) or the brain section with anti-ICAM-1 (MAb YN1/1.7) almost completely abolished binding of SLN to inflamed vessels in the CNS (Figure 6).

Discussion

Migration through the structurally unique endothelial BBB represents an essential step in the trafficking of lymphocytes from the blood into the CNS parenchyma during an inflammatory process. The adherence of circulating lymphocytes to cerebral endothelium thereby represents the first step in lymphocyte extravasation and is mediated at least in part by the interaction of CAMs expressed on endothelium and lymphocytes. In order to understand the specific mechanism of recruitment of lymphocytes into the CNS during chronic inflammation, we chose to investigate the expression and function of CAMs during the first stage of chronic inflammation in EAE in the SJL/J mouse.

We observed upregulation of endothelial ICAM-1 and to a lesser extent of VCAM-1 in the CNS of SJL/J mice afflicted with EAE. Expression of ICAM-1 has been observed on inflamed venules during EAE[28,51]; however, this study demonstrates for the first time the expression of VCAM-1 on venules in the CNS of mice afflicted with EAE. We could not demonstrate the endothelial expression of either the mucosal addressin MAdCAM-1 nor the peripheral addressin PNAd. Expression of both addressins have been demonstrated in other inflammatory situations such as the inflamed pancreas of the nonobese diabetic mouse,[52] where expression of PNAd and MAdCAM-1 occurs in parallel with the presence of L-selectin- and β7-integrin-positive lymphocytes in the pancreatic infiltrate. Additionally, ICs in EAE brains did not stain for L-selectin, which however does not completely rule out a possible role for L-selectin in the recruitment of ICs into the CNS, as L-selectin is a rapidly-downregulated molecule.[53]

The majority of inflammatory cells within the CNS parenchyma stained brightly for α4-integrin in both aEAE and tEAE. Staining for the β7-integrin with a panel of anti-murine β7 MAbs detected only scattered cells. This suggests that most of the α4-integrin-positive inflammatory cells express $\alpha4\beta1$-integrin rather than $\alpha4\beta7$-integrin. The additional lack of staining with the MAb DATK 32, which specifically recognizes β7-integrin in context with α4-integrin, is consistent with this finding. Importantly, we have observed a similar phenotype of brain ICs by flow cytometry in a different model of CNS inflammation induced by focal *Corynebacterium parvum* injection. (Engelhardt et al., manuscript in preparation) Since no MAb directed against the murine β1-subunit was available to us, we cannot completely exclude the possibility of an unknown β-chain being paired with α4-integrin at this time. The relative paucity of cells bearing $\alpha4\beta7$-integrin in the CNS parenchyma, however, suggests that the subpopulation of lymphocytes that home to the mucosal associated lymphoid tissues is not recruited significantly to the CNS during the chronic inflammatory stage. Consistent with this we could not detect the expression of the $\alpha4\beta7$-integrin ligand MAdCAM-1 in inflamed EAE brains. Induction of MAdCAM-1 expression has been described on vessels in the spinal cord of Biozzi mice during the late stage of relapsing clinical EAE.[28] The difference in mouse strains or the disease model might explain the discrepancy of these data with our findings. In accordance with our data, Cannella et al reported the expression of endothelial MAdCAM-1 only during relapsing stages of the clinical EAE in the SJL/J mouse.[51] The lack of MAdCAM-1 expression therefore argues that it plays no role in the initial development of the disease.

ICs, staining brightly for α4-integrin, were present at a time when VCAM-1 was upregulated on endothelium in the CNS of mice suffering from EAE. By performing adhesion assays on frozen sections of EAE brains, we could demonstrate that VCAM-1 mediated adhesion of mononuclear cells to inflamed endothelium in EAE brains. Since the activity of β2-integrins has been reported to be temperature-sensitive,[54,55] the binding assays investigating the interaction of endothelial VCAM-1 with α4-integrin ligands on mononuclear cells were performed at 4 C.

Jurkat cells expressing $\alpha4\beta1$- but no $\alpha4\beta7$-integrin avidly bound to venules on frozen sections of EAE brains but not to those of control brains. Our functional studies suggest that the adhesion of Jurkat cells was mediated by the interaction of endothelial VCAM-1 with $\alpha4\beta1$-integrin on the Jurkat cell.

Adhesion assays performed with lymph node cells derived from inflamed peripheral lymph nodes demonstrated that they bind to VCAM-1 preferentially via $\alpha4\beta1$-integrin rather than $\alpha4\beta7$-integrin, as MAbs directed against α4-integrin but not against β7- or $\alpha4\beta7$-integrin-inhibited binding. $\alpha4\beta7$-Integrin can support interactions with VCAM-1 on CNS venules, however, as the $\alpha4\beta7^{high}$ $\alpha4\beta1^{-}$ TK1 cells selectively adhered to vessels in inflamed brains but not to vessels in control brains. Our studies showed that this interaction involves VCAM-1 and $\alpha4\beta7$-integrin at 4 C. The ability of TK1 cells to bind may reflect the very high levels of $\alpha4\beta7$-integrin, which are greater than the levels on naive T cells, or may indicate some activation of TK1 $\alpha4\beta7$-integrin, as $\alpha4\beta7$ binds VCAM-1 preferentially after activation.[19,24] VCAM-1 expressed on cerebral vessels during EAE can therefore support adhesion of mononuclear cells by both $\alpha4\beta1$- and $\alpha4\beta7$-integrin. The lack of β7-positive cells in the inflammatory infiltrate, as well as our results with lymph

node cells, however, suggest that $\alpha 4\beta 1$-integrin dominates the interaction with VCAM-1 in EAE.

Both $\alpha 4\beta 1$-integrin and $\alpha 4\beta 7$-integrin have been shown to mediate cell attachment to the alternatively spliced domain CS1 of fibronectin, which is a site distinct from the fibronectin-binding site of VLA-5.[56] Our results do not favor the involvement of fibronectin-binding activity in adhesion to EAE vessels, as the $\alpha 4\beta 1$-mediated adhesion of Jurkat cells, the $\alpha 4\beta 7$-mediated binding of TK1 cells, and the binding of lymph node cells was completely inhibited by an MAb directed against VCAM-1 at 4 C.

In this study we observed a dramatic upregulation of ICAM-1 on endothelium in the CNS in both aEAE and tEAE animals, and it was additionally present on ICs. Our results are consistent with expression patterns found in EAE in the mouse by others who demonstrated the presence of ICAM-1 both on endothelium within lesions and in uninvolved areas in addition to the expression of ICAM-1 on the ICs.[27,28,51] These studies, however, did not compare the induction of endothelial ICAM-1 in aEAE *versus* tEAE. We observed a striking difference of expression of ICAM-1 in the two models. Whereas in tEAE vascular expression of ICAM-1 was dramatically upregulated on venules, in aEAE ICAM-1 expression could also be detected on capillaries throughout the CNS. Since Lee and Olitzky[57] reported that aEAE could be induced in mice with greater efficiency and frequency when *B. pertussis* vaccine was added to the immunization protocol, the administration of *B. pertussis* organisms has been standard procedure in the murine EAE models. In our hands, the injection of heat-inactivated *B. pertussis* organisms alone was sufficient to induce expression of ICAM-1 on CNS capillaries, suggesting this to be a possible mechanism by which *B. pertussis* injection facilitates the development of CNS inflammation and thus leads to an increase in the severity of clinical EAE.

To investigate the functional importance of endothelial ICAM-1, we performed binding assays with mononuclear cells on frozen sections of EAE brains at 20 C. MAbs directed either against LFA-1 or ICAM-1 showed slightly inhibitory effects already at 4 C. At 20 C however, binding of lymph node cells isolated from inflamed lymph nodes could be very effectively inhibited by either MAb, suggesting that the interaction of LFA-1 on mononuclear cells with endothelial ICAM-1 may also play a role in recruitment of lymphocytes to the CNS during the first chronic inflammatory stage of EAE in the SJL/J mouse.

The first *in vivo* studies applying MAbs directed against $\alpha 4$-integrin or ICAM-1, are consistent with our findings, namely that both VLA-4/VCAM-1 and LFA-1/ICAM-1 might play a role in the development of CNS inflammation during EAE.[25,26,27] Pointing out the importance of certain adhesive pathways, these previous studies could not rule out the possibility that the *in vivo*-applied mAbs actually altered an aspect of the immune response different from lymphocyte migration through the BBB in a way that the animals did not develop EAE. Our results provide further evidence that, indeed, the LFA-1/ICAM-1 and $\alpha 4$-integrin/VCAM-1 interaction at the level of the BBB is critical for the recruitment of inflammatory lymphocytes into the CNS and therefore for the development of EAE.

In conclusion, our results provide evidence that at least two adhesion pathways mediated via endothelial ICAM-1 and VCAM-1 may support binding of circulating cells to cerebral endothelium via their known counterreceptors LFA-1 and $\alpha 4\beta 1$-integrin, respectively. The presence of LFA-1 and $\alpha 4$-integrin positive inflammatory cells in the CNS of diseased animals, and the demonstrable participation of these pathways in endothelial cell adhesion, strongly argue for a functional role of endothelial ICAM-1 and VCAM-1 for the recruitment of ICs during the first stage of chronic inflammation in EAE.

It is unlikely, however, that endothelial expression of ICAM-1 and VCAM-1 alone controls the recruitment of ICs to the CNS, since a significant proportion of vessels immunoreactive for ICAM-1 or VCAM-1 was not associated with an inflammatory cell infiltrate. The expression of additional endothelial adhesion receptors or the local presence of specific chemoattractants might explain our findings.[58] Recent models of leukocyte-endothelial interaction as multistep process suggest that the presence or absence of particular chemokines[59] or other lymphocyte-subset-specific chemoattractants, may be an initial determinant of lymphocyte-subset recruitment as well. This could help to explain the relative paucity of ICs within the CNS parenchyma expressing $\beta 7$-, αE- or $\alpha 6$-integrin phenotypes, preferentially associated with IC in the gut or skin, respectively.[30,31,48]

As EAE still serves as our best animal model for human inflammatory demyelinating diseases of the CNS such as multiple sclerosis, the understanding of the functional importance of CAMs expressed in the CNS during EAE may provide valuable insights for the design of new treatment strategies that can be applied to humans.

References

1. Wekerle H, Engelhardt B, Risau W, Meyermann R: Passage of lymphocytes across the blood-brain bar-

rier in health and disease. Pathophysiology of the Blood-Brain Barrier: Edited by BB Johansson, C Owman, H Widner. New York, Elsevier Science Publishers, 1990, pp 439–445
2. Reese TS, Karnovsky MJ: Fine structural localization of a blood-brain barrier to exogenous peroxidase. J Cell Biol 1967, 34:207–217
3. Crone C, Olesen SP: Electrical resistance of brain microvascular endothelium. Brain Res 1982, 241:49–55
4. Coomber BL, Stewart PA: Morphometric analysis of CNS microvascular endothelium. Microvasc Res 1985, 30:99–115
5. Brown AM, McFarlin DE: Relapsing experimental allergic encephalomyelitis in the SJL/J mouse. Lab Invest 1981, 45:278–285
6. Raine CS, Barnett LB, Brown A, Bhar T, McFarlin DE: Neuropathology of experimental allergic encephalomyelitis in inbred strains of mice. Lab Invest 1980, 43: 150–157
7. Raine CS: Multiple Sclerosis: Pathology, Diagnosis, and Management. Edited by J Hallpike, CWM Adams, WW Tourtelotte. London, Chapman & Hall, 1983
8. Alvord EC, Jr, Kies MW, Suckling AJ (Eds): Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis. New York, AR Liss, 1984
9. Zamvil SS, Steinman L: The T lymphocyte in experimental allergic encephalomyelitis. Annu Rev Immunol 1990, 8:579–621
10. Fritz RB, Chou C-HJ, McFarlin DE: Induction of experimental allergic encephalomyelitis in PL/J and (SJL/J × PL/J) F1 mice by myelin basic protein and its peptides: localization of a second encephalitogenic determinant. J Immunol 1983, 130:191–197
11. Petinelli CB, McFarlin DE: Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after in vitro activation of lymph node cells by myelin basic protein: Requirement for $LYT1^+2^-$ T lymphocytes. J Immunol 1981, 127:1420–1423
12. Cross AH, Cannella B, Brosnan CF, Raine CS: Homing to central nervous system vasculature by antigen-specific lymphocytes: localization of ^{14}C-labeled cells during acute, chronic, and relapsing experimental allergic encephalomyelitis. Lab Invest 1990, 63:162–170
13. Trotter J, Steinman L: Homing of $Lyt\text{-}2^+$ and $Lyt\text{-}2^-$ T cell subsets and B lymphocytes to the central nervous system of mice with acute experimental allergic encephalomyelitis. J Immunol 1984, 132:2919–2923
14. Wekerle H, Linington C, Lassmann H, Meyermann R: Cellular immune reactivity within the CNS. Trends Neurosci 1986, 9:271–277
15. Wekerle H, Sun D, Oropeza-Wekerle RL, Meyermann R: Immune reactivity in the nervous system: modulation of T-lymphocyte activation by glial cells. J Exp Biol 1987, 132:43–57
16. Shimizu Y, Newman W, Gopal TV, Horgan KJ, Graber N, Beall D, van Seventer GA, Shaw S: Four molecular pathways of T cell adhesion to endothelial cells: Roles of LFA-1, VCAM-1, and ELAM-1 and changes in pathway hierarchy under different activation conditions. J Cell Biol 1991, 113:1203–1212
17. Osborn L: Leukocyte adhesion to endothelium in inflammation. Cell 1990, 62:3–6
18. Carlos TM, Schwartz BR, Kovach NL, Yee E, Rosa M, Osborn L, Chi-Rosso G, Newman B, Lobb R, Harlan JM: Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. Blood 1990, 76:965–970
19. Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, Weissman IL, Hamann A, Butcher EC: $\alpha4\beta7$ integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. Cell 1993, 74: 185–195
20. Vadas MA, Gamble JR, Smith WB: Regulation of myeloid blood cell-endothelial interaction by cytokines. Adhesion: Its Role in Inflammatory Disease. Edited by JM Harlan, DY Liu. New York, Freeman and Company, 1992, pp 65–81
21. Pober JS, Gimbrone MAS Jr., Lapierre LA, Mednrick DL, Fiers W, Rothlein R, Springer TA: Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor and immune interferon. J Immunol 1986, 137:1893–1896
22. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC: Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transedothelial migration of human neutrophils in vitro. J Clin Invest 1989, 83:2008–2017
23. Hemler ME, Elices MJ, Parker C, Takada Y: Structure of the integrin VLA-4 and its cell-cell and cell-matrix adhesion functions. Immunol Rev 1990, 114:45–65
24. Ruegg C, Postigo AA, Sikorski EE, Butcher EC, Pytela R, Erle DJ: Role of integrin alpha4 beta7/alpha4 beta p in lymphocyte adherence to fibronectin and VCAM-1 and in homotypic cell clustering. J Cell Biol 1992, 117: 179–189
25. Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N: Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha4\beta1$ integrin. Nature 1992, 356:63–66
26. Baron JL, Madri JA, Ruddle NH, Hashim G, Janeway CA, Jr.: Surface expression of $\alpha4$ integrin by CD4 T cells is required for their entry into brain parenchyme. J Exp Med 1993, 177:57–68
27. Archelos JJ, Jung S, Mäurer M, Schmeid M, Cassmann H, Tawatani T, Miyasaka M, Toyka KV, Hartung HP: Inhibition of experimental autoimmune encephalomyelitis by an antibody to the intercellular adhesion molecule ICAM-1. Ann Neurol 1993, 34:145–154
28. O'Neill JK, Butter C, Baker D, Gschmeissner SE, Kraal G, Butcher EC: Expression of vascular addressins and ICAM-1 by endothelial cells in the spinal cord during chronic relapsing experimental allergic encephalomyelitis in the Biozzi AB/H mouse. Immunol 1991, 72:1–6
29. Holzmann B, McIntyre BW, Weissman IL: Identification of a murine Peyer's patch-specific lymphocyte homing receptor as an integrin molecule with an alpha chain homologous to human VLA-4. Cell 1989, 56:37–46
30. Miyake K, Weissmann IL, Greenberger JS, Kincade PW: Evidence for a role of the integrin VLA-4 in lympho-hemopoiesis. J Exp Med 1991, 173:599–607

31. Miyake K, Medlin K, Ishihare K, Kimot M, Auerbach R, Kincade PW: A VCAM-like adhesion molecule on murine bone marrow stromal cells mediates binding of lymphocyte precursors in culture. J Cell Biol 1991, 114:557–565
32. Birkeland ML, Metlay J, Sanders VM, Fernandes-Botran R, Vitetta ES, Steinman RM, Pure E: Epitopes on CD45R (T200) molecules define differentiation antigens on murine B and T lymphocytes. J Mol Cell Immunol 1988, 4:71–85
33. Imhof BA, Ruiz P, Hesse B, Palacios R, Dunon D: EA-1, a novel adhesion molecule involved in the homing of progenitor T lymphocytes to the thymus. J Cell Biol 1991, 114:1069–1078
34. Kilshaw PJ, Baker KC: Unique surface antigen on intraepithelial lymphocytes in the mouse. Immunol Lett 1988, 18:149–154
35. Kilshaw PJ, Murant SJ: Expression and regulation of $\beta 7$ (p) integrins on mouse lymphocytes: relevance to the mucosal immune system. Eur J Immunol 1991, 21: 2591–2597
36. Campanero MR, Pulido R, Ursa MA, Rodriquez-Moya M, Landazuri MO, Sanchez-Madrid F: An alternative leukocyte homotypic adhesion mechanism, LFA-1/ICAM-1-independent, triggered through the human VLA-4 integrin. J Cell Biol 1990, 110:2157–2165
37. Gallatin MW, Weissman IL, Butcher EC: A cell-surface molecule involved in organ-specific homing of lymphocytes. Nature 1983, 303:30–34
38. Streeter PR, Rouse BT, Butcher EC: Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. J Cell Biol 1988, 107:1853–1862
39. Briskin MJ, McEvoy LM, Butcher EC: The mucosal vascular addressin, MAdCAM-1, displays homology to immunoglobulin and mucin-like adhesion receptors and to IgA. Nature 1993, 363:461–464
40. Andrew DP, Berlin C, Hamann A, Kilshaw P, Butcher EC: Function of the lymphocyte homing receptor defined by a novel $\alpha 4\beta 7$ specific mAb (submitted)
41. Duijvestijn AM, Kerkhove M, Bargatze RF, Butcher EC: Lymphoid tissue and inflammation-specific endothelial cell differentiation defined by monoclonal antibodies. J Immunol 1987, 138:713–719
42. Jalkanen S, Bargatze RF, Herron LR, Butcher EC: A lymphoid cell surface glycoprotein involved on endothelial cell recognition and lymphocyte homing in man. Eur J Immunol 1986, 16:1195–1202
43. Bargatze RF, Wu NW, Weissman IL, Butcher EC: High endothelial venule binding as a predictor of the dissemination of passaged murine lymphomas. J Exp Med 1987, 166:1125–1131
44. Stamper HB, Woodruff JJ: Lymphocyte homing into lymph nodes: in vitro demonstration of the selective affinity of recirculatin lymphocytes for high endothelial venules. J Exp Med 1976, 144:828–834
45. Naparstek Y, Holoshitz J, Eisenstein S, Reshef T, Rapaport S, Chemke J, Ben-Nun A, Cohen IR: Effector T lymphocyte cells migrate to the thymus and persist there. Nature 1982, 300:262–264
46. Sriram S, Solomon D, Rouse RV, Steinman L: Identification of T cell subsets and B lymphocytes in mouse brain experimental allergic encephalitis lesions. J Immunol 1982, 129:1649–1651
47. Traugott U, Raine CS, McFarlin DE: Acute experimental allergic encephalomyelitis in the mouse: immunopathology of the developing lesion. Cell Immunol 1985, 91:240–254
48. Mackay CR, Marston WL, Dudler L, Spertini O, Tedder TF, Hein WR: Tissue-specific migration pathways by phenotypically distinct subpopulations of memory T cells. Eur J Immunol 1992, 22:887–895
49. Zeine R, Owens T: Direct demonstration of the infiltration of murine central nervous system by Pgp-1/$CD44^{high}CD45RB^{low}CD4^{+}$T cells that induce experimental allergic encephalomyelitis. J Neuroimmunol 1992, 40:57–70
50. Lee WT, Vitetta ES: The differential expression of homing and adhesion molecules on virgin and memory T cells in the mouse. Cell Immunol 1991, 132:215–222
51. Cannella B, Cross AH, Raine CS: Adhesion-related molecules in the central nervous system. Upregulation correlates with inflammatory cell influx during relapsing experimental autoimmune encephalomyelitis. Lab Invest 1991, 65:23–31
52. Hänninen A, Taylor C, Streeter PR, Stark LS, Sarte JM, Shizuru JA, Simell O, Michie SA: Vascular addressins are induced on islet vessels during insulitis in NOD mice and are involved in lymphoid cell binding to islet endothelium. J Clin Invest 1993, 92:2590–2515
53. Kishimoto TK, Jutila MA, Berg EL, Butcher EC: Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. Science 1989, 245: 1238–1240
54. Marlin SD, Springer TA: Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function associated antigen-1 (LFA-1). Cell 1987, 51:813–819
55. Rothlein R, Springer TA: The requirement for lymphocyte function associated antigen 1 in homotypic leukocyte adhesion stimulated by phorbol ester. J Exp Med 1986, 163:1132–1149
56. Wayner EA, Garcia-Pardo A, Humphries MJ, McDonald JA, Carter WG: Identification and characterization of the T lymphocyte adhesion receptor for an alternative cell attachment domain (CS-1) in plasma fibronectin. J Cell Biol 1989, 109:1321–1330
57. Lee JM, Olitzky PK: Simple method of enhancing development of acute disseminated encephalomyelitis in mice. Proc Soc Exp Biol Med 1955, 89:263–267
58. Oppenheim JJ, Zachariae COC, Mukaida N, Matsushinia K: Properties of a novel proinflammatory supergene "Intercrine" cytokine family. Annu Rev Immunol 1991, 9:617–648
59. Butcher EC: Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell 1992, 67:1033–1036